Original Article

Cloning and Expression of Helicobacter Pylori UreB122 (a Segment of the B-subunit of Urease Gene)

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Abstract

Introduction: Helicobacter pylori is associated with the chronic gastritis, peptic ulcer, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Moreover, antibiotic therapies do not protect against potential re-infection while have risk for development of drug resistance. Therefore, vaccine-mediated protection against H. pylori became an attractive clinical interest. H. pylori urease plays an important role in survival and pathogenesis of the infection. In this study the UreB122 (aa143-264) gene cloned in pET expression vector and the recombinant protein (rUreB122) was over expressed in Escherichia coli (E. coli .(

Patients and Methods: Genomic DNA of the standard H. pylori strain 26695 was isolated as the template and UreB122 gene was amplified by PCR. Prokaryote expression vector pET32a was inserted with UreB122 gene (pET32a-UreB122). The recombinant plasmid was used to transform competent E. coli DH5α, and positive clones were selected. Then the recombinant plasmid was used to transform E. coli BL21DE3 for expression of recombinant protein UreB122. The expression of recombinant protein was induced by isopropythio-β-D-galctoside (IPTG) at different concentration and examined by SDS-PAGE. Western blot assay was used to determine immunoreactivity of rUreB122 by anti His-Tag antibodies against recombinant UreB122 .

Results: In comparison with the reported corresponding sequences, the nucleotide sequence homology of UreB122 gene was 99.9%. UreB122 fusion protein was able to react with the anti His-Tag antibody .

Conclusion: A prokaryotic expression system with high efficiency of H. pylori UreB122 gene was successfully established and UreB122 fusion protein showed satisfactory immunoreactivity. These results indicate that production of specific recombinant protein is an alternative and potentially more expeditious strategy for development of H. pylori vaccine.

Keywords: Helicobacter pylori, urease, recombinant protein, pET32a.

Introduction

Helicobacter pylori is a gram-negative microaerophilic bacterium, which colonizes the stomach. It is the causitive agent of gastritis, peptic ulcer disease and a risk factor for B cell mucosa-associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma (1-3). The high prevalence of H. pylori infection and the emergence of antibiotic resistant strains led to studies on H. pylori vaccine (1, 4). Additionally, natural H. pylori infection does not provide protective immunity, and re-infection occurs occasionally (5, 6). The bacterium is able to withstand the harsh acidic environment of the stomach by producing several factors including urease. Urease is essential for H. pylori colonization of gnotobiotic piglets, cynomolgus monkeys and mice (7, 8).

The antigenic potential of urease has been demonstrated in several studies (9-13). The urease of H. pylori is composed of two distinct subunits, UreA (26.5 KDa) and UreB (61.7 KDa), of which the UreB is considered as an excellent antigen and the active site of the bacterium (9, 13-15). Based on structural and bioinformatics analysis of UreB, we determined a region consisting of 122 aa (UreB122), constructed recombinant plasmid containing ureB122 gene and examined expression of recombinant UreB122 peptide.

Patients and Methods

Bacterial strains and plasmid: The PCR experiments were performed with genomic DNA prepared from H. pylori, ATCC 26695. Escherichia coli (E. coli) DH5□α and E. coli BL21DE3 were used for cloning and expression experiments. Plasmid pET-32a was used as cloning and expression vector.

Amplification of ureB122 gene: H. pylori ATCC 26695 was grown on H. pylori-selective agar plates with 10% defibrillated sheep blood and antibiotics at 37°C under microaerobic conditions (10% CO2, 5% O2, and 85%N2). Genomic DNA was extracted by routine phenol-chloroform method (16). The DNA fragment coding for UreB122 (nucleotides of 922-1287) gene was amplified using oligonucletides UreB1 (5'-ATATGGATCCATGCAACAAATCCCTACAGC-3') as the forward primer with an endonuclease site of BamHI and UreB2 (5'-ATTTAAGCTTTTAAGCTGCCATAGTG TCTTCC-3') as the reverse primer with an endonuclease site of HindIII. Amplification was made in a total volume of

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50µl of reaction mixture containing 10µl of 10× PCR buffer, 2.5 mM Mgcl2, 200µM dNTP, 1.25 units of Taq polymerase, 20 pmol of each primer and 1µl of sample DNA under these conditions: 94 °C for 5 min, then 30 cycles at 94°C for 1min, 58°C for 1min, 72°C for 1 min, and 10 min at 72°C. The PCR products were analyzed on 1% agarose gels stained with ethidium bromide. The expected size of target amplification fragment was 365bp.

Cloning and sequencing: Fragments of BamHI and HindIII-digested ureB122 were inserted into the BamHI/HindIII site of expression vector pET-32a through enzyme digestion and legation reactions. Then the recombinant plasmid pET-32a-ureB122 was confirmed by PCR and restriction enzyme digestion. Recombinant pET-32a-ureB122 was amplified in competent E. coli DH5□α, and then was extracted by Sambrook's methods (16). The resulting plasmid pET-32a-ureB122 was transformed into competent final host E. coli BL21DE3, and kanamycin resistance used for selection (16). A large scale of recombinant plasmid was prepared and identified by restriction enzymes. DNA sequencing was performed by a DNA automatic sequencer.

Expression and identification of the fusion protein: The UreB122 expression system pET-32a-ureB122-BL21DE3 was cultured in lysogeny broth (also called Luria broth) (LB) medium at 26°C and induced by isopropylthio-□-D-galactoside (IPTG) at different concentrations of 0.5, 1 and 1.5 mmol/L. The precipitate was isolated by centrifugation and cell pellet was broken by 100 µl loading buffer. The molecular weight of UreB122 fusion protein was identified by separation of whole-cell lysates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Immunoblot analysis: The immunoreactivity of UreB122 fusion protein was determined by Western blotting for which proteins were transferred to nitrocellulose sheets, and incubated with anti His-Tag antibody and HRP-labeling sheep anti-rabbit IgG as the first and second antibodies, respectively

Results

Construction of recombinant pET-32a-ureB122: The PCR product amplified from genomic DNA of H. pylori strain 26695 is shown in Figure 1.

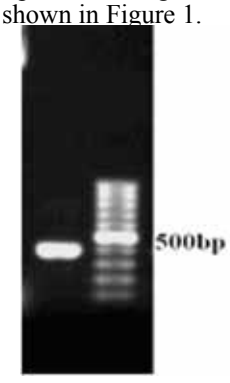


Figure 1: The target fragment of ureB122 gene amplified from H. pylori strain 26695. Lane1: 100bp DNA marker, Lane2: the target amplification of ureB122gene.

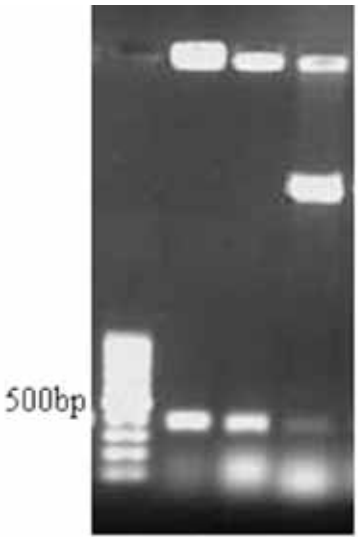
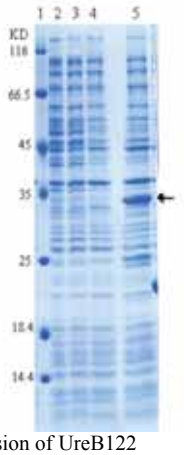


Figure 2: Agarose gel electrophoresis analysis of recombinant pET-32a-ureB122. Lane1: 100bp DNA marker, Lane2: PCR product of ureB122gene, Lane 3: Double digest of PCR product with BamHI and HindIII, Lane4: Double digest of recombinant pET-32a-ureB122 with BamHI and HindIII

Nucleotide sequence analysis: The ureB122 gene nucleotide sequence in the recombinant plasmid vector of pET-32a-ureB122 was consistent with that of H. pylori ureB published in the gene bank. The homologies of the nucleotide sequences in the pET-32a-ureB122 compared with the published ureB122 gene sequences were 99.9% (17).

Expression of recombinant fusion protein: The recombinant pET-32a-ureB122 was transformed into BL21 E. coli strains, and the fusion protein was expressed. The pET-32a is designed for cloning and high-level expression of peptide sequences fused with the 109aa Trx•Tag™ thioredoxin protein. Therefore, the 1.5 mmol/L of IPTG was able to efficiently induce the expression of UreB122 fusion protein with predicated molecular masses of 34KD (Fig. 3).



Cloning and Experssion of UreB122

Fig.3: Expression of UreB122 fragment in pET-32a-ureB122-BL21. Lane1: Protein marker, Lane2: Bacterial cell without plasmid, Lanes 3 and 4: Non-induced recombinant bacterial cells, Lane5: Induced bacterial cells (4h).

Western blotting: The recombinant fusion protein was recognized by anti His-Tag antibody against the N-terminal of fusion protein. Analysis of recombinant UreB122 fusion protein by Western blotting was shown in Fig. 4.

Figure 4 Western blotting of expressed pET-28a-UreB122 products. Lane1: Induced recombinant bacterial cells, Lane2: Protein marker.

Discussion

H. pylori makes a urease in abundance (10-15% of total protein by weight), which hydrolysis the urea into ammonium and enables the bacterium to withstand within gastric lumen (7). This enzyme also can stimulate the innate and specific immunity response. Since the urease of H. pylori is a high molecular weight (530 KDa) multimeric enzyme, in this study, we chose the UreB subunit which accounts as an active site and a bi-nickel center near the active site (15).

Based on bioinformatics analysis, we determined a 122 aa (143-264) fragment of UreB (Fig. 5) that is functionally important for urease activity and also has antigenic epitopes (14).In our study, the UreB122 gene from H. pylori 26695 was amplified by PCR. PCR product was cloned in pET-32a and then transformed into E. coli BL21. The UreB122 gene cloned in this study, showed high homologies of nucleotide sequences compared with the published corresponding sequences (17). In this study, SDS-PAGE demonstrated that the constructed prokaryotic expression pET-32a- UreB122-BL21 efficiently produces rUreB122 at the 1.5 mmol/L of IPTG. The anti His-Tag antibody against N-terminal (six Histidine) of recombinant protein could recognize and combine with UreB122 recombinant protein which was confirmed by Western blot.

Recently, epitope-based approaches represent an alternative to immunization with whole-cell vaccine or subunit vaccine, particularly when the natural infection did not lead to protective immunity (18). The persistence of H. pylori in the host stomach indicated that immune responses against the bacterium were not favorable to eradicate the infection. In this study, we postulated that truncated UreB can be able to trigger the immune response different from the native protein. As previously reported, the CD4+ T cells (Th cells) are responsible for the protective immunity against H. pylori (19). Shi et al. (20) identified three functional Th cell epitopes (U546-561, U229-244, and U237-251) in UreB protein. Peptides U546-561, and U229-244 were restricted Th2 type epitopes, and U237-251 restricted Th1 epitopes. Our UreB122 (aa 143-264) recombinant protein contains two Th cell epitopes; peptide U229-244 (Th2 type), and U237-251 (Th1 type). In addition, Li et al (21) identified two B-cell epitopes in UreB, U200-230, and U211-225 which were also found in our recombinant UreB122.In conclusion, UreB122 recombinant protein can be potentially evoke CD4+ T cell and B cell responses, and a UreB122 expression system with high efficiency has een successfully constructed in our study.

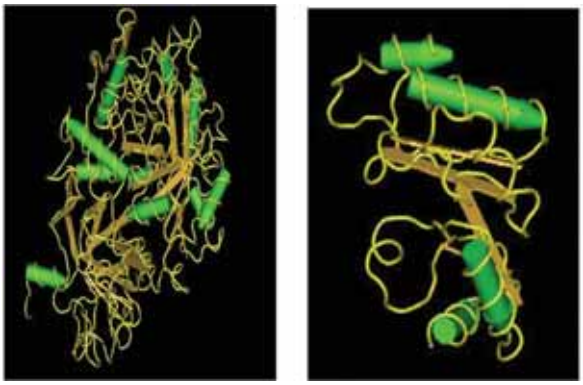


Fig.5. The three-dimensional model of H. pylori UreB (left) and UreB122 fragment (right).

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